AD_____

AWARD NUMBER: W81XWH-04-1-0158

TITLE: Functions and Mechanisms of Sleep in Flies and Mammals

PRINCIPAL INVESTIGATOR: Michael Rosbash, Ph.D.

CONTRACTING ORGANIZATION: Brandeis University
Waltham, MA 02454

REPORT DATE: October 2009

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
1 October 2009	Final	15 Jan 2004 – 18 Sep 2009

4. TITLE AND SUBTITLE

Functions and Mechanisms of Sleep in Flies and Mammals

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-04-1-0158

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Michael Rosbash, Ph.D.

E-Mail: rosbash@brandeis.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Brandeis University
Waltham, MA 02454

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Work on sleep at Brandeis focuses on Drosophila melanogaster as well as the more traditional rodent models. The Drosophila works aims to exploit the genetic advantages of this organism yet still learn about aspects of sleep relevant to humans. The major finding has been that the human therapeutic Carbamazapine is a potent sleep-deprivation agent in flies. Current data indicate that its effects are mediated through the Rdl GABAA receptor, which has implications for the role of this drug in humans. One of the rodent laboratories is focused on the regulation of sleep and waking in the basal forebrain. The goal is to identify gene expression changes in its cholinergic neuronal subset, and specific neuron purification has been accomplished. Another rodent laboratory is studying the effects of sleep deprivation on the intrinsic electrophysiology and gene expression properties of neocortical neurons. Interesting changes in firing properties of layer 5 pyramidal neurons have been observed, and gene expression assays from these cells are underway. The final two projects involve the role of sleep in homeostatic plasticity and fear conditioning. These are being done both in vivo, in freely behaving animals, and "ex vivo, in cortical slices after sleep deprivation or training.

15. SUBJECT TERMS
Sleep, Learning, Gene Regulation, Neuron Plasticity

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	32	19b. TELEPHONE NUMBER *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

General Introduction

There were eight PIs over the past few years at Brandeis with a common interest in sleep. During the past few years, Dr. Katz's (Aim 6) research interests have lead away from sleep leaving seven labs dedicated to sleep investigation. Although the investigators run autonomous research programs with individual reports, every research program has interactions with one or more other of the other laboratories. This is a characteristic of Brandeis, i.e., close and productive inter-laboratory collaborations. Indeed, nearly all publications on sleep from Brandeis reflect collaborations between two or more laboratories as we are a highly interactive research community.

Finally, I emphasize that the sleep community at Brandeis is still relatively young. Most of us have no long-term track record in this field and have become interested in sleep only in the past several years. So we all began this work de novo. As a consequence, the group is still only now entering a mature phase. As stated, much of the work initiated during the grant should reach fruition (i.e., publication) over the next few years.

Aims 1 & 2: To determine the role played by Drosophila clock neurons in regulating sleep and to identify other groups of neurons in the Drosophila brain involved in regulating sleep and wakefulness (Griffith & Rosbash).

Introduction

Drosophila exhibits a sleep-like state, previously referred to as "rest," that shares multiple characteristics with human sleep (Hendricks et al., 2000; Shaw et al., 2000). Flies maintained in standard 12:12 light: dark (L:D) cycles have sustained periods of sleep, which occur predominantly during the dark period for female flies (Shaw et al., 2000). The effects of many sleep modulating compounds are thought to be conserved between flies and humans (Hendricks et al., 2000; Shaw et al., 2000), but there is little current understanding of the circuitry that drives sleep in Drosophila. Consistent with Drosophila's contribution to the understanding of many biological phenomena, the study of sleep in this model system will undoubtedly provide insight into human sleep and its regulation. Flies therefore provide a useful model system not only to dissect the genetic and biochemical underpinnings of sleep drive but also as a platform to screen for novel sleep and vigilance drugs.

Body

1) Role of clock neurons in sleep architecture.

In flies as well as humans, sleep occurs in a circadian manner, with more sleep during the night than during the day. There are, however, almost no experiments that link the well-studied circadian system of Drosophila to sleep. Moreover, little is known about the circuitry underlying the generation or timing of sleep in flies. To determine if the cells that make up the core of the circadian clock were important for the generation of sleep, as opposed to just regulation of its timing, we expressed the cell death gene hid under control of cry-GAL4. This GAL4 driver expresses in almost all of the cells of the clock, and elimination of these cells with HID renders flies completely arrhythmic. Examination of sleep in these flies indicates that while the timing of sleep is disrupted, the total amount of sleep remains the same. These results suggest that the circadian clock regulates the timing of sleep, but is not critical for its generation in either L:D or D:D conditions.

Because cry-GAL4; UAS-HID genotype eliminates many different kinds of clock cells, the failure to see an effect on total sleep could reflect some compensation or balancing of effects. Therefore, we expressed transgenes encoding the Shaw potassium channel or Shaw RNAi, in much more defined subsets of the clock circuit. Expression of Shaw, which is a leak channel, would be expected to block firing while expression of Shaw RNAi will enhance firing in cells whose resting potential is regulated by endogenous Shaw (Hodge et al., 2005). Animals expressing tim-GAL4; UAS-Shaw or tim-GAL4; UAS-Shaw/pdf-GAL80 fall asleep earlier in both the day and night. These data suggest that sleep latency is controlled by the dorsal group of clock neurons, including LN_ds and DNs. Flies expressing pdf-GAL4; UAS-ShawRNAi have significantly less sleep in both the day and the night. These results are consistent with the

activity of LN_vs normally acting to inhibit sleep, both the amount of total sleep as well as the timing of sleep onset (sleep latency).

Our work on role of the LN_vs and their control light by GABAergic inputs was published in PNAS (Shang et al., 2008) and Neuron (Parisky et al., 2008). Work on dorsal clock neurons is ongoing.

2) GABAergic circuits that control sleep.

In humans, both onset and maintenance of sleep is regulated by GABAergic transmission, and many of the important therapies for sleep disorders modulate GABA receptors. We have defined a role for the $GABA_A$ receptor RDL in fly sleep. We have begun to define the circuits in which this receptor acts. Expression of the Shaw potassium channel under control of GAD2B-GAL4 significantly reduces both daytime and nighttime sleep. This suggests that inhibition of GABAergic transmission, by decreasing excitability of these neurons, is enough to disrupt total sleep. Using fragments of the GAD promoter, we have subdivided these neurons and we find that there are both sleep- and wake-promoting GABAergic cells. We have identified a subset that innervate LN_vs and we see putative wake-promoting cells that project to the region of the dorsal clock cells. This fits with the idea that the clock has a role in promoting both the sleeping and waking states.

3) Role of the $GABA_A$ receptor subunit RDL in sleep.

We have recently published a paper (Agosto et al., see below) on the role of $GABA_A$ receptors in regulating sleep onset and sleep maintenance in Drosophila. This paper also encompasses the work described in the section above and the section below. The paper describes strong evidence that these two ubiquitous features of sleep, sleep onset and sleep maintenance, can be assayed in flies and are affected by $GABA_A$ receptors like in mammals. However, the two systems have major difference in regulation. This conclusion stems from experiments examining fly strains with $GABA_A$ receptor subunit (Rdl) mutants, with altered kinetics of desensitization. The analysis of sleep onset and sleep maintenance in these strains shows that sleep onset is affected more strongly by the mutants affecting RDL desensitization kinetics. We interpret these results to indicate that there are major differences in the circuits that regulate sleep onset and sleep maintenance. These differentially exploit the biophysical properties of the RDL receptors, to provide behavioral complexity. Given the pivotal role of the $GABA_A$ receptor complex in the pharmacological treatment of several neurological disorders as well as sleep, we propose that targeting specific aspects of $GABA_A$ receptor function such as its desensitization kinetics is a potential route for the design of drugs to target differentially specific aspects of sleep such as only sleep onset.

4) Effect of carbamazepine on fly sleep.

Based on a connection between human circadian rhythms and manic-depressive (bipolar) disorder, we began an investigation of whether therapeutic drugs for this disease, especially those with no known or well-agreed upon target, might have an effect

in flies. The purpose was to use Drosophila genetics and identify the missing, behaviorally relevant, drug-target. We were most keen on lithium, but this drug turned out to have only a modest and difficult to track phenotypic effect. In contrast, carbamazepine (CBZ), a drug used for bipolar disorder and even more frequently for epilepsy, dramatically reduces sleep and increases locomotor activity. This is surprising, since its effect on humans suggests that it should have the opposite effect on sleep, namely, act as a hypnotic. To address this paradox, we assayed the physiological effects of the drug on Drosophila RDL and human $GABA_A$ channels in the Xenopus oocyte system. The results were interesting and consistent with an opposite effect on flies and human $GABA_A$ receptors. CBZ exerts its effect on the fly RDL channel by enhancing channel desensitization. The Rdl^{A302S} mutant channel, which desensitizes poorly, is resistant to CBZ. Importantly, flies carrying this mutation are partially resistant to the behavioral effects of CBZ, specifically to the potent effects of CBZ on sleep onset. This supports the idea that desensitization of RDL is critical for sleep onset.

5) Screen for other brain regions that affect sleep.

We have recently undertaken a GAL4 screen to identify cells whose neuronal activity regulates the structure and/or amount of sleep. We expressed dTrpA1, a warmth-activated cation channel under control of over 50 GAL4 lines to screen a wide variety of brain areas. The amount and quality of sleep were assessed at both 21^{o}C (channel inactive) and 30^{o}C (channel causes depolarization of neurons). While still preliminary, the results of this screen indicate that many brain areas can drive alterations in sleep structure, but many fewer can alter the total amount of sleep. One interpretation of these data is that there are many areas that have an impact on sleep, but the homeostat causes these changes to become compensated. The few areas that when activated can alter total sleep are candidate regions that control this homeostatic set point. These studies will provide an important foundation for a global understanding of sleep regulation.

Key Research Accomplishments

- Defining an effect of GABAergic cells in sleep.
- Defining a role for the $GABA_A$ receptor Rdl in sleep latency and sleep maintenance in Drosophila.
- Defining an effect of the drug carbamazepine on fly sleep.
- Defining a role for the circadian neurons in sleep.
- Identification of additional brain regions involved in sleep control and homeostasis.

Reportable Outcomes

PDF cells are a GABA-responsive wake-promoting component of the Drosophila sleep circuit. Parisky, K.M., Agosto, J., Pulver, S., Shang, Y., Kuklin, E., Hodge, J.L., Kang, K., Liu, X., Garrity, P., Rosbash, M., Griffith, L.C. Neuron. 2008 Nov 26; 60(4):672-82. Erratum in: Neuron. Jan 15; 61(1):152 (2009).

Light-arousal and circadian photoreception circuits intersect at the large PDF cells of the Drosophila brain. Shang, Y., Griffith, L.C., Rosbash, M. Proc Natl Acad Sci U.S.A. 2008 Dec 16; 105(50):19587-94. Epub Dec 5 (2008).

Modulation of $GABA_A$ receptor desensitization uncouples sleep onset and maintenance in Drosophila. Agosto, J., Choi, J.C., Parisky, K.M., Stilwell, G., Rosbash, M., Griffith, L.C. Nat Neurosci. 2008 Mar; 11(3):354-9. Epub Jan 27 (2008).

Sleep: hitting the reset button. Griffith, L.C. and Rosbash, M. Nat. Neurosci. 11:123-124 (2008).

Conclusion

We have been able to combine genetics and pharmacology to address sleep in Drosophila. The results indicate that $GABA_A$ receptors and clock neurons are important in ways that resemble their contributions to mammalian sleep. We have also defined a role for carbamazepine, which might help address its unknown mechanism of action as a human therapeutic. Finally, we are at the beginning stages of examining GABAergic cells, which have not been very well studied in the fly system. Given the prominent role of the GABA receptors, we anticipate that these inhibitory cells will be important and we intend to define their relationship to the clock-sleep neuronal system. Finally, we have undertaken a more global screen for additional brain regions that control sleep with the goal of understanding it at the circuit level.

References

Hamasaka, Y., Wegener, C., and Nassel, D.R. (2005). GABA modulates Drosophila circadian clock neurons via GABAB receptors and decreases in calcium. J Neurobiol 65, 225-240.

Hendricks, J.C., Finn, S.M., Panckeri, K.A., Chavkin, J., Williams, J.A., Sehgal, A., and Pack, A.I. (2000). Rest in Drosophila is a sleep-like state. Neuron 25, 129-138.

Hodge, J.J., Choi, J.C., O'Kane, C.J., and Griffith, L.C. (2005). Shaw potassium channel genes in Drosophila. J Neurobiol 63, 235-254.

Pitman, J.L., McGill, J.J., Keegan, K.P., and Allada, R. (2006). A dynamic role for the mushroom bodies in promoting sleep in Drosophila. Nature 441, 753-756.

Shaw, P.J., Cirelli, C., Greenspan, R.J., and Tononi, G. (2000). Correlates of sleep and waking in Drosophila melanogaster. Science 287, 1834-1837.

Griffith Personnel

Jose L. Agosto: Graduate Student
Anna Chiau: Undergraduate student
Choong-Kwan Choi: Graduate Student
Nathan Donelson: Postdoc
Hannah Janoowalla: Undergraduate student
Bumsook Lee: Undergraduate student
Katherine M. Parisky: Postdoc
Nicolas Pirez: Postdoc
Stefan Pulver: Graduate student
Jie Shi: Undergraduate student

Rosbash Personnel

Jose L. Agosto: Graduate student
Erica Hinteregger: Undergraduate student
Sebastian Kadener: Postdoc
Elzbieta Kula-Eversole: Postdoc
Emi Nagoshi: Postdoc
Anna Sivachenko: Postdoc

Aim 3: Regulation of basal forebrain cholinergic gene expression during sleeping and waking (Birren).

Introduction

The mammalian basal forebrain is a key regulatory system for the control of sleeping and waking. Cholinergic neurons in this region form part of a complex neural circuit that includes inhibitory GABAergic neurons (Berger-Sweeney et al., 2001). The cholinergic and GABAergic neurons have opposing actions on sleep pathways, with cholinergic neurons showing activity during waking and REM sleep (Detari et al., 1984; Szymusiak and McGinty, 1986; Detari and Vanderwolf, 1987), and GABAergic neurons having increased activity during non-REM sleep (Pollock and Mistlberger, 2003; Sanford et al., 2003). The GABAergic neurons also regulate the activity of the cholinergic neurons in the circuit, further contributing to the regulation of sleep-wake cycles (Vazquez and Baghdoyan, 2003). Thus the balance of cholinergic and GABAergic neurons in the basal forebrain, and the patterns of gene expression in these different populations, are of critical importance for the regulation of sleep and waking patterns. Over the course of this project we have developed methods that allow the identification and isolation of cholinergic neurons in the adult mouse basal forebrain, defined cell-specific gene expression patterns for these neurons during sleep deprivation, and, over the past year, have used this information to begin to investigate the mechanisms that set the balance of sleep and wake-associated neurons and neuronal activity patterns. As we continue this research our goal is to understand how neuronal cell type-specific properties are established and contribute to awareness states.

Body

Over the course of this project we have developed methods to allow the neuronal cell type-specific analysis of basal forebrain neurons. We have used this approach to examine patterns of gene expression in cholinergic neurons that are associated with higher firing rates in the waking animal. By comparing gene expression in cholinergic neurons isolated from both sleep deprived and control mice, we have defined a set of genes associated with the waking state in these neurons. We have continued this study by further investigating the role of one factor, brain-derived neurotrophic factor (BDNF), which has been identified as a sleep-regulated gene by several laboratories including our own. We have shown that neurotrophic factors, including BDNF, regulate cholinergic and GABAergic development and synapse formation, suggesting a model for balancing the activity basal forebrain circuits. Together, these experimental approaches are defining the functional properties of sleep-regulated genes in setting the activity levels of neuronal populations that control patterns of sleeping and waking.

We investigated genes involved in sleep regulation using microarray screens of basal forebrain cholinergic neurons derived from sleep deprived and control mice. To carry out these experiments we established a sleep deprivation paradigm that uses a slowly rotating wheel system that minimizes interventional and handling stress while obtaining significant sleep deprivation over a period of hours. We took advantage of the

selective expression of the p75 low affinity neurotrophin receptor on the basal forebrain cholinergic neurons (Lin et al., 2007) to isolate the cholinergic neurons from these mice using fluorescence-activated cell sorting (FACS) and used the RNA isolated from those neurons to probe DNA microarrays. Analysis of this microarray data has shown that sleep deprivation results in large changes in expression in a number of genes. In triplicate experiments we identified 1500 genes showing greater than two-fold changes following sleep deprivation including genes encoding ion channels, receptors, and activity-dependent proteins. Interestingly, we did not identify BDNF as being regulated in the cholinergic neurons, although in earlier experiments we had identified it as being regulated by sleep deprivation in total basal forebrain samples. This suggests that BDNF is regulated by sleep deprivation in a neuron-specific manner in non-cholinergic neurons of the basal forebrain. These experiments are permitting us to define a dynamic pattern of neuron-specific gene expression across a neural circuit that regulates sleep-wake cycles.

We investigated the regulation of the basal forebrain cholinergic and GABAergic neurons that regulate sleeping and waking by examining the role of BDNF in the development of neuronal properties and synaptic regulation. We found that BDNF contributed to the regulation of GABAergic and cholinergic neuron number and that signaling through the p75 neurotrophin receptor provides a mechanism for signaling between different neuron types to set the number of neurons in basal forebrain circuits *in vitro* and *in vivo* (Lin et al., 2007). Over the past year we have defined a role for BDNF and the p75 receptor in determining the timing and number of GABAergic and cholinergic synapses that form between basal forebrain neurons. We believe that these mechanisms play a role in setting activity levels in basal forebrain circuits and have recently begun to identify activity-dependent genes in the basal forebrain. One of the candidate genes that we have identified as being activity dependent in this system is TrkB, the receptor for BDNF. Thus, these experiments are beginning to define the regulatory mechanisms that control the development and function of sleep-regulated basal forebrain circuits.

Key Research Accomplishments

- Established cultures and defined markers for GABAergic and cholinergic neurons in the basal forebrain.
- Identified p75 as a specific marker of cholinergic neurons within the basal forebrain.
- Used high through-put fluorescence-activated cell sorting for the isolation of purified populations of cholinergic basal forebrain neurons.
- Establishment of a mouse sleep deprivation model that controls for handling stress.
- Carried out sleep deprivation protocols on adult mice and isolated cholinergic neurons from basal forebrain for sleep deprived and control animals.
- Triplicate microarray screens for sleep deprived and control basal forebrain cholinergic neurons.
- Identification and validation of cholinergic gene expression changes of candidate genes.

- Isolation of cholingeric neurons and microarray analysis of gene expression regulation during recovery sleep.
- Identification of neurotrophins as factors that control the balance of basal forebrain cholinergic and GABAergic neurons.
- Analysis of activity-dependent gene expression in basal forebrain neurons.
- Defined p75-dependent regulation of cholinergic and GABAergic synapses in the basal forebrain.

Reportable Outcomes

Lin PY, Hinterneder JM, Rollor SR, Birren SJ. Non-cell-autonomous regulation of GABAergic neuron development by neurotrophins and the p75 receptor. J Neurosci 27:12787-12796 (2007).

Conclusion

The cholinergic and GABAergic neurons of the basal forebrain form a key neural circuit for the regulation of sleep patterns. We have begun to dissect the cholinergic-GABAergic neural circuit by examining sleep-regulated genes in a single population of cholinergic neurons. These experiments have identified a panel of genes that are regulated by sleep deprivation and that are allowing us to investigate the how specific genes regulate the activity properties of subsets of neurons that have firing properties associated with the sleeping or waking state. We plan to continue our investigation of the role of BDNF and other sleep and activity-regulated genes in the regulation of GABAergic and cholinergic synaptic and activity properties.

References

Berger-Sweeney J, Stearns NA, Murg SL, Floerke-Nashner LR, Lappi DA, Baxter MG (2001) Selective immunolesions of cholinergic neurons in mice: effects on neuroanatomy, neurochemistry, and behavior. J Neurosci 21:8164-8173.

Detari L, Vanderwolf CH (1987) Activity of identified cortically projecting and other basal forebrain neurones during large slow waves and cortical activation in anaesthetized rats. Brain Res 437:1-8.

Detari L, Juhasz G, Kukorelli T (1984) Firing properties of cat basal forebrain neurones during sleep-wakefulness cycle. Electroencephalogr Clin Neurophysiol 58:362-368.

Lin PY, Hinterneder JM, Rollor SR, Birren SJ (2007) Non-cell-autonomous regulation of GABAergic neuron development by neurotrophins and the p75 receptor. J Neurosci 27:12787-12796.

Pollock MS, Mistlberger RE (2003) Rapid eye movement sleep induction by microinjection of the GABA-A antagonist bicuculline into the dorsal subcoeruleus area of the rat. Brain Res 962:68-77.

Sanford LD, Tang X, Xiao J, Ross RJ, Morrison AR (2003) GABAergic regulation of REM Sleep in reticularis pontis oralis (RPO) and caudalis (RPC) in rats. J Neurophysiol.

Szymusiak R, McGinty D (1986) Sleep-related neuronal discharge in the basal forebrain of cats. Brain Res 370:82-92.

Vazquez J, Baghdoyan HA (2003) Muscarinic and GABAA receptors modulate acetylcholine release in feline basal forebrain. Eur J Neurosci 17:249-259.

Birren Personnel

Susan Birren: Faculty
Lanhua Came: Postdoc
Abby Finkelstein: Summer undergraduate
Jeanine Hinterneder: Graduate student, Postdoc
Jason Luther: Postdoc, Research Associate
Tatyana Pozharskaya: Research Technician
Nithya Setty: Undergraduate student

Aim 4: Effects of sleep deprivation on neuronal physiology and gene expression (Nelson).

Introduction

The initial objective of this project was to identify changes in electrophysiology and gene expression in neocortical neurons following Sleep Deprivation (SD). One of the main problems that confounded earlier SD studies was the poor control of physical stress experienced by the animals because of the sleep deprivation techniques (like chronic muscle stress induced by requiring animals to stay on small platforms floating in water in the "flower pot" technique or forced locomotion on a rotating cylinder partially submerged in water) (Vertes, 2004; McDermott et al. 2003). Secondly, gene expression studies of sleep were limited by the use of tissue homogenates that dilute changes in gene expression occurring in specific cell types. In order to circumvent this problem we developed a novel technique for isolating fluorescently labeled neurons and extracting and amplifying their RNA. Technical development of the deprivation method and the RNA isolation method were successful, however, it became clear that sleep deprivation produced only modest changes in gene expression in cortical neurons, and that the major electrophysiological changes we observed were attributable to stress. Therefore, we adopted the additional aim of characterizing the physiology and gene expression of brain stem neurons in the locus coeruleus, a nucleus that plays a critical role in the control of wakefulness.

Body

In year 1, we fabricated an apparatus for performing the disk-over-water (DOW) technique (Rechtschaffen, A., et al., 1999) for SD. This technique causes minimal physical stress and equalizes stress for the control and experimental mice. The apparatus consists of a plexi-glass disk that extends in two adjacent plastic cages. The cages are filled with water to a small height below the disk. The disk is mounted on a motor that can be rotated in a random direction using a computer. The control and the experimental mice are implanted with pairs of EEG and EMG electrodes that are used to monitor gross electrical activity of the brain and neck muscle tone, respectively. The recordings are analyzed in real time and the disk is rotated whenever the experimental mouse falls asleep. This causes both the mice to walk in the direction opposite to the rotation of the disk to avoid falling in water. The control mouse can sleep when the experimental mouse is awake. According to our preliminary recordings, the control mouse is deprived of about 30% of its sleep while the experimental mouse is deprived of 75-85% of its sleep. Food and water are available ad-libitum. The animals are maintained on 12 hr:12 hr light-dark cycle.

Baseline awake-sleep pattern is recorded for 24 hrs from both the animals after they have recovered from electrode implantation surgery and have acclimatized to the electrode wires and the cage. If the electrode-tissue connections are good, we see a bimodal distribution of EEG and EMG activity from which the awake-sleep thresholds are determined. If the standard deviation of EMG is greater than the threshold value, the animal is considered to be awake. If it is less, then if mean power in the Delta frequency

band is higher than the threshold, NREM is scored. If both EMG & Delta power are low, then the mean power in the Theta band is checked. If it is higher than the threshold, REM is scored; otherwise the animal is considered to be awake but quiet. The visually determined state of the mice matches with the state determined by the above algorithm with high probability. The propensity of sleep as scored by above algorithm, increases during light cycle and decreases during dark cycle. Recordings also showed a small increase in the duration of NREM sleep of the experimental mouse after SD for 24 hours. This was also reflected in an increase in power in the delta frequency band compared to baseline.

The mice used in above studies express a genetically encoded fluorescent protein in a sub-population of neurons allowing us to repeatedly select an identified population of cortical neurons for studying gene expression following SD (Feng et al., 2000). We carried out whole-cell recordings from the fluorescently labeled layer 5 pyramidal neurons of the primary motor cortex in slices from experimental and control mice (YFP-H: mouse line)[2]. Our initial assay of spontaneous AP (action-potential) firing showed a variable decrease in firing frequency in the sleep-deprived mice (Control = 0.4 +/- 0.19 Hz, Sleep-deprived = 0.16 +/- 0.07) . To find out whether the difference in spontaneous activity was due to different synaptic activity/connections or due to difference in intrinsic properties, we measured neuronal firing in response to different levels of current injection in the presence of synaptic blockers. We observed that neurons from sleep-deprived animals fired at lower frequencies and had higher minimum current thresholds for evoking firing. The RMP (resting membrane potential) and voltage threshold for evoking firing were not significantly different. However, we observed that the total membrane conductance (measured at RMP) was higher in sleep deprived mice than in control mice (Control = 98 +/-5 S/F, Sleep-deprived = 124 +/- 8 S/F; conductance normalized by capacitance). These results have been presented at the annual Society for Neuroscience Meeting and were selected for presentation in the "Neuroscience in Sleep and Circadian Biology DataBlitz", an ancillary event of the Annual Meeting organized by the NIH National Center on Sleep Disorders Research, and the NIH Sleep Disorders Research Advisory Board.

We then set out to investigate the underlying ion channels that might contribute to the increase in conductance upon sleep deprivation. Hyper-polarization activated mixed cation conductance, I_h, and inwardly rectifying potassium conductance K_{IR} were isolated but were not found to be significantly different between the control and sleep deprived mice. Further experiments showed no significant differences in intrinsic excitability or membrane conductance between sleep deprived and yoked control mice after 24 hour sleep deprivation. However, both these groups had significantly increased membrane conductance (normalized to capacitance) compared to cage control animals (cage control: 91+/- 6 S/F, yoked control: 106 +/- 4 S/F, sleep-deprived: 103 +/-3 S/F). Our interpretation of these results is that the variable increase in membrane conductance in our sleep deprivation experiments may be caused by stress due to restricted mobility (McDermott, et al., 2003) and/or to surgical implantation of recording electrodes for monitoring EEG and EMG. It is possible that the sleep-deprived mice were more stressed than the control mice in our preliminary experiments in which we observed an increase in membrane conductance and a higher current threshold for action potential firing.

Unfortunately, gene expression analysis was also relatively unrevealing. In a pair of experiments comparing expression in pyramidal neurons from the cortices of deprived and yoked controls, 30 transcripts were differentially expressed by 1.5 to 2.5 fold, but no obvious pattern of affected genes was apparent, and false positives could not be rigorously excluded. We feel confident that we have excluded the possibility of huge changes in gene expression in these neurons following 24h sleep deprivation, but the confound of stress may have made more modest changes difficult to detect.

We next focused our efforts on a neural cell type known to be involved in the regulation of sleep and alertness, the noradrenergic neurons of the locus coeruleus (LC). This brain stem nucleus projects widely throughout the brain, and is implicated in the modulation of sleep/wake states and vigilance. LC neuronal activity is strongly regulated across the sleep/wake cycle. We obtained mice that express GFP under the control of the tyrosine hydroxylase promoter. This allows us to unambiguously identify the catecholaminergic neurons in the LC in brain slice physiology experiments and to isolate them for gene expression experiments. Our initial experiments have focused on characterizing the baseline electrophysiology of these neurons and the compliment of ion channels that they express. Although intrinsic electrophysiological properties of these neurons have previously been studied, little is known about the specific ion channels that mediate those properties. These channels may represent important targets for regulating alertness.

LC neurons have depolarized resting membrane potentials (between -40 to -50 mV) and fire spontaneous action potentials in the slice at frequencies ~ 0.2 – 5Hz. We carried out cell-type specific mRNA profiling and found that LC neurons express a very different set of channels from those we have previously identified in cortical and hippocampal pyramidal neurons and interneurons. Specifically, we have found that LC neurons lack expression of all but one splice variant of the KCNQ subunits that mediate the M-current. Physiologically they lack an M-current defined with a pharmacological blocker (XE-991) and this may contribute to their depolarized resting membrane potential. These neurons are also unusual in that they lack the HCN1 subunit that contributes in most other neurons studied to the hyperpolarization-activated cation current. Physiologically LC neurons lack a postsynaptic I_h, although this current can also be expressed in axon terminals where it presynaptically regulates transmitter release. If this is the case in LC neurons, it is due to expression of the HCN2 and HCN3 subunits. We have also identified a calcium channel regulatory subunit that is selective expressed in LC neurons. We hypothesize that this could be important in regulating the substantial resting calcium current that these neurons are known to possess, that may contribute to their spontaneous activity. In order to test this we obtained knock-out animals produced by Verrity Letts at Jackson labs. Unfortunately, no difference in LC spontaneous activity was observed in the knock out mice indicating either that this subunit is not important for the resting calcium current, or that there has been some other compensation in these animals. Finally, we also examined the ligand gated receptors expressed by these neurons. We have identified the serotonin receptor likely to mediate the known interaction between the serotonergic Raphe nucleus and the LC (Htr1d). We have also confirmed the prior observation that LC neurons lack the alpha1 subunit of the GABA-A

receptor and have found that this is correlated with GABA-A mediated IPSCs that have slow kinetics, as expected from other systems. A paper describing these correlated gene expression and physiological results is in preparation.

We also began a collaboration with Jeff Agar to examine protein level expression in LC neurons. These neurons express a rich complement of peptides at the prepro RNA level. We have facilitated Jeffs proteomic experiments on LC neurons in brain slices and will help him correlate RNA and protein expression of peptides in these neurons.

Key Research Accomplishments

- Designed and built apparatus to implement DOW sleep deprivation technique in mice.
- Perfected hardware/software for monitoring and scoring sleep/wake EEG and EMG.
- Perfected RNA isolation, amplification and hybridization from cortical layer 5 pyramidal neurons for analysis of cell-type specific gene expression.
- Identified physiological consequences of deprivation on firing properties of layer 5 pyramidal neurons in mouse motor cortex.
- Carried out initial RNA isolation, amplification and hybridization experiments from cortical layer 5 pyramidal neurons for analysis of deprivation-induced changes in cell-type specific gene expression.
- Found that previously observed physiological consequences of deprivation on firing properties of layer 5 pyramidal neurons in mouse motor cortex were most likely due to stress of deprivation.
- Carried out RNA isolation, amplification and hybridization experiments from cortical layer 5 pyramidal neurons, and found that deprivation-induced changes in gene expression in this cell type are modest.
- Studied the baseline physiological properties of Locus Coeruleus (LC) neurons known to be important in regulating sleep and vigilance.
- Used cell-type specific expression profiling to identify multiple channels specifically present and absent from LC neurons that contribute to their unique physiological properties.

Reportable outcomes

The following were primarily supported by other funding sources, but were also partially supported by the present grant as they represent necessary steps in perfecting the gene expression assays used in carrying out the research goals.

- Abstract: Sugino K., Hempel C.M., Miller M., Hattox, A., Huang, Z.J., and Nelson S.B. A Global view of the molecular identity of cortical cell types. Soc. Neurosci. Abst. 611.13 (2004).
- Sugino K, Hempel CM, Miller M, Hattox AM, Shapiro P, Wu C, Huang ZH and Nelson SB. Molecular taxonomy of major neuronal classes in the adult mouse forebrain. Nat. Neurosci. 9:99-107 (see also News&Views p.10-12) (2005).

- Hempel C.M., Sugino K. and Nelson S.B. A manual method for the purification of fluorescently labeled neurons from the mammalian brain. Nature Protocols. 2:2924-9 (2007).

The following abstract reported our progress towards understanding the electrophysiological consequences of sleep deprivation on cortical function in mice.

- Abstract: Taneja, P., et al., Effects of sleep deprivation on electrophysiological properties and gene expression in mouse neocortex. Abstract Viewer/Itinerary Planner, Washington, DC: Society for Neuroscience, Program No. 500.13, 2005 (Online).

The following represent collaborative projects applying methods for cell-type-specific gene expression analysis to understand circadian rhythms in flies.

- Kadener S., Menet J., Sugino K., Horwich M.D., Nawathean P., Vagin V.V., Zamore P.D., Nelson S.B. and Rosbash M. A role for microRNAs in the *Drosophila* circadian clock. Genes Dev. 23:2179-91 (2009).

- Nagoshi E., Sugino K., Kula E., Okazaki E., Tachibana T., Nelson S.B., and Rosbash M. (2009) Gene expression dissection of the circadian neuronal circuit of drosophila. Nature Neurosci. In Press.

The following manuscripts are in preparation:

- Wong J.H., Boggio K.J., Zaia N.M., Lazarus R., Sugino K., Nelson S.B., Agar N.Y.R., and Agar J.N. (2009) In situ MALDI mass spectrometry imaging at cellular resolution. Nature Meth. In Preparation

- Hempel C.M. Taneja, P., Sugino K., and Nelson S.B. Physiological genomics of locus coeruleus neurons. J. Neurosci. In Preparation.

Conclusion

We identified a reproducible physiological change in the firing properties of a major class of output neuron of the cerebral cortex in sleep deprived mice. Unfortunately, changes in the physiology and gene expression of neocortical pyramidal neurons are largely attributable to the effects of stress. Basic physiological properties of locus coeruleus neurons and the specific ion channels that mediate them have been identified. These studies have identified multiple potential targets for manipulations of alertness in mammals.

References

- Feng, G., et al., Imaging neuronal subsets in transgenic mice expressing multiple spectral variants of GFP. Neuron 28(1): p. 41-51 (2000).

- McDermott, C. M., et al. Sleep deprivation causes behavioral, synaptic, and membrane excitability alterations in hippocampal neurons." J Neurosci 23(29): 9687-95 (2003).
- Rechtschaffen, A., et al., Effects of method, duration, and sleep stage on rebounds from sleep deprivation in the rat. Sleep. 22(1): p. 11-31 (1999).
- Rechtschaffen, A., et al., Physiological Correlates of Prolonged Sleep Deprivation in Rats. Science 221(4606): p. 182-184 (1983).
- Vertes, R.P., Memory consolidation in sleep; dream or reality. Neuron 44(1): p. 135-48 (2004).

Personnel

Chris Hempel: Postdoc
Praveen K. Taneja: Postdoc
Jason Wu: Undergraduate student

Aim 5: Role of Sleep in Homeostatic Plasticity (Turrigiano).

Introduction

The initial goal of this Aim was to examine the role of sleep in homeostatic cortical plasticity. More recently, we realized that differences in cortical circuitry due to circadian cycle are even more profound than those due to sleep, and so have switched focus to examine the effects of circadian cycle on cortical microcircuitry and plasticity. Our recent data raise the very surprising possibility that cortical circuitry is rewired on an hour by hour basis by change in circadian cycle. We are very excited by this finding and are continuing to pursue it actively. We began by studying this process in freely behaving animals using chronically implanted electrode arrays. This turned out to be technically difficult in young animals, so more recently we have adopted an "ex vivo" approach pioneered in my lab, in which animals are first manipulated and then brain slices are made to examine changes in cortical microcircuitry. The ex vivo approach has proved to be very fruitful and this is the focus of our current research.

Body

In vivo approach

We have had numerous technical difficulties with our attempts to record cortical activity in freely behaving juvenile rats, largely because we are trying to record from superficial cortical layers of young animals. We had to reduce the size and weight of the arrays to implant on the small animals without affecting their ability to move freely. We now have a working electrode design, but have run into significant problems getting stable recordings from implanted animals, because for the younger age animals we know the brain is still growing. While we have had some success the yield (number of neurons we can stably record from) has been low. In order to move to older animals, we have had to first show that homeostatic plasticity is present in older (adult size) animals. We have now verified that homeostatic plasticity is present in upper layers of cortex in older animals, so the next step is to try implanting our modified electrodes into older animals with slower growth. We are continuing with this approach, but at a reduced level of effort. Don Katz continues to serve in an advisory role.

Ex vivo approach to study circadian influences on cortical connectivity

As an alternative to the *in vivo* approach, we are now taking an ex-vivo approach that has been very successful in my lab; this was pioneered by my postdoc Arianna Maffei with partial support from this grant, and has resulted in several publications (Maffei et al., 2006, 2008). In collaboration with Sacha Nelson's lab we found that sleep deprivation depresses cortical function as assayed ex vivo in mouse motor cortex, and my postdoc Kiran Nataraj found a similar depression of cortical activity in visual cortex. Upon further analysis, however, it appeared that many of these effects could be attributed to stress or to circadian cycle, rather than sleep effects. This lead us to think more deeply about the possibility that cortical synaptic connections could be rapidly altered by the circadian cycle. The idea that modulatory influences might have rapid effects on cortical function is not new; what we realized, however, is that things like light/dark cycles can very rapidly influence the strengths of cortical connections, and in particular the balance between excitation and inhibition, independently of modulatory influences. This raised

the intriguing possibility that the basic set of connections in a local cortical network might be fundamentally different during the day and night, which could in turn have major implications for behavior. To test this idea my postdoc Biljana Djukic tried the very simple experiment of cutting cortical slices during different phases of the circadian cycle, and determining the excitability of the local network (after modulatory influences had been washed away).

To assess the possible effect of circadian rhythm on neuronal activity within the monocular visual cortex, she compared spontaneous firing rates of layer 5 pyramidal neurons at two opposing circadian time points: subjective mid-day at T6 and mid-night at T18. Our initial finding indicated a 3.67 fold decrease in the firing rate at T18 (0.06±0.03, n=32) as compared to that at T6 (0.22±0.07, n=35). However, pyramidal neuron population within layer 5 contains two subpopulations of cells termed thin-tufted and thick-tufted based on their morphological characteristics. After further analysis and thorough cell classification, our data indicates that only thick-tufted cells show a marked decrease in their firing rates at night (T6: 0.44± 0.14, n=15; T18: 0.03±0.01, n=14), while thin-tufted cells appear to maintain constant firing rate regardless of circadian time (T6: 0.06±0.02, n=20; T18: 0.09±0.05, n=18). Change in neuronal firing activity can arise from changes in intrinsic excitability and/or changes in the excitatory and inhibitory synaptic drive. Assessment of intrinsic properties was carried out by generating firing rate vs. current (FI) curves in the presence of synaptic blockers. No significant difference was found in the resting membrane potential (T6: -65.48±0.58, n=22; T18: -65.31±0.71, n= 20), input resistance (T6: 59.57±3.59; 52.92±2.27), firing threshold (T6: -36.28±0.55; T18: 36.82±0.86) or slope of the FI curve (T6: 75.31±4.52; T18: 75.31±3.77) in thick-tufted layer 5 pyramidal neurons at T18 compared to T6. To test whether decreased firing activity at T18 could be explained by changes in synaptic drive, we first looked at non-evoked synaptic activity by recording miniature excitatory and inhibitory postsynaptic currents (mEPSC, mIPSC), followed by a study of spontaneous (evoked + non-evoked) synaptic activity by comparing total inhibitory and excitatory charge. None of the measured parameters (amplitude, frequency, rise and decay time) of mEPSCs and mIPSCs were significantly different between the two circadian times. However, there was a 76 % increase in total inhibitory synaptic charge (T6: n= 11; T18, n=15; p=0.064), and a 7 % decrease in excitatory synaptic charge (T6: n=12; T18: n=14; p=0.601). Collectively our results suggest that within layer 5 of the monocular visual cortex thick-tufted pyramidal cells selectively display decreased firing activity during the subjective night (T18), in part due to receiving increased inhibitory synaptic input.

We are currently doing a number of additional experiments to verify that these differences are due to circadian cycle, rather than light/dark cycle. First, we have found that 6 additional hours of light or dark does not convert subjective day firing patterns into subjective night (and vice versa), suggesting that the effects we see are due to circadian changes. Second, Kiran Nataraj has found that prolonged visual deprivation modulates the intrinsic excitability of Layer 5 pyramidal neurons, an effect that is distinct from the effects of circadian cycle (which affects inhibition but not intrinsic excitability). Third, we are in the process of analyzing a non-rhythmic circadian mutant mouse (bmal) to verify that the difference between T6 and T18 is gone in this mouse. Third, we are

examining a diurnal rodent (the Nile rat) to ask whether the effects we see track wake/rest cycles (rather than light/dark cycles) and are inverted in a diurnal rodent.

Further, we are working on the mechanism that produces these profound changes in cortical activity. We have traced the differences to changes in the amount of inhibition these neurons receive, raising the possibility that inhibitory plasticity is regulated by circadian cycle. Our data represent the first demonstration that circadian rhythms rewire cortical circuitry on an hour-by-hour basis, and suggest that our brain circuitry is in fundamentally different states at different times of day. These data are likely to have very important implications for understanding mammalian behavior.

Key Research Accomplishments

- Miniaturization and modification of electrode arrays for young rats in upper cortical layers; this will likely prove useful for a number of future studies.
- Demonstration that homeostatic plasticity operates in adult animals in upper layers (layer 2/3).
- Found that neurons in layer 5 (the output layer of cortex) have their intrinsic excitability modulated by visual experience, so that output can be turned on or off.
- Demonstration that cortical microcircuitry is rapidly (within hours) rewired by the phase of the circadian cycle. This is our most important finding, and forms the basis of ongoing studies.

Reportable Outcomes

We submitted a manuscript to Neuron on the role of intrinsic plasticity in layer 5 visual cortex (Kiran Nataraj first author). Following review we have performed a number of additional experiments and plan to have this manuscript re-submitted within the next month.

Biljana Djukic has a manuscript in preparation on the effects of circadian cycle on cortical microcircuitry. We expect to have a manuscript submitted within the next few months.

Conclusions

We have found that cortical microcircuitry is rapidly re-wired by circadian cycle. This finding has important implications for mammalian behavior, and we are currently working out the mechanism by which these effects are exerted.

Personnel

Lanhua Came: Postdoc
Biljana Djukic: Postdoc
Arianna Maffei: Postdoc
Kiran Nataraj: Postdoc
Corette Wierenga: Postdoc

Aim 7- Mass Spectrophotometry Aim: To examine qualitative and quantitative protein expression changes during sleep and wake (Agar).

Introduction

Dr. Jeffrey Agar was hired at Brandeis in June 2005 as an Assistant Professor of Chemistry. His research focuses on mass spectrometry, a technique that will augment research on the mechanisms of sleep and complement microarray gene expression and bioinformatics research.

Body

Since his hire less then four years ago, initial priorities were to remodel laboratory space, choose the proper mass spectrometry equipment, and then install and make this equipment functional. These goals have been met and the laboratory has been fully operational for over one year now. A post doctoral fellow, Ravi Madulla, was hired and is spearheading collaborations between the Agar laboratory and both the Rosbash and Nelson laboratories to study sleep in flies and mice, respectively. In these studies, transgenic mice and flies that contain specific neurons that are labeled with fluorescent proteins are used. The fluorescently labeled cells are used as spatial markers, to pinpoint locations within the brain. These studies take advantage of MALDI imaging capabilities, which allow protein imaging with 100 micrometer resolution, approaching the size of large cells and certainly making possible the observation of important changes within small brain regions. This approach is particularly well-suited for assaying changes in neuropeptide composition in defined brain nuclei, which accompany sleep-deprivation for example. For future experiments, regions of the brain known to be integral to the sleep process, such as the SCN, will also be dissected from awake and asleep mice, and changes to peptides and proteins that result from sleep will be characterized. This approach will also be coupled with neuron sorting as described above in other Aims, and preliminary results on LC neurons are promising.

Current studies involve the determination of the Drosophila melanogaster neuropeptidome involved in circadian rhythm as elucidated by mass spectrometry. Through the use of mass spectrometry, we have determined neuropeptides that are regulated by the presence and/or absence of a circadian neuropeptide. Results for this are promising and have been presented at three conferences: 1) The Brandeis Neurogenetics Celebration, 2) The 57 th Annual ASMS Conference on Mass Spectrometry and Allied Topics, and 3) The 18 th Annual International Mass Spectrometry Conference. Two manuscripts related to these results are currently in preparation. The ultimate goal of these studies is to search for major changes in protein (neuropeptide) composition within brain regions important for sleep regulation as a function of sleep versus wake or as a function of sleep deprivation.

Key Research Accomplishments

Three manuscripts and 1 book chapter have been published by the Agar group. Two manuscripts that relate to sleep studies are currently in preparation. Posters and oral presentations in regards to sleep studies have been presented and are listed under the Reportable Outcomes section.

Conclusion

This mass spectrometry laboratory has a number of unique capabilities and is among the most advanced in the world. We anticipate that imaging and peptide analysis, combined with the neuronal purification skills of the other sleep laboratories, will give rise to important results over the next year or two. We have developed techniques that allow for the detection of neuropeptides (manuscripts in preparation) both from intact brains with minimal sample preparation and from single neurons.

Reportable Outcomes

Protein Aggregation and Protein Instability Govern Familial ALS Patient Survival. Q. Wang, J. L. Johnson, N. Y.R. Agar, and J. N. Agar. PLoS Biology. 6 (7) e170 (2008).

A Hierarchical Algorithm for Calculating the Entire Isotopic Fine Structures of Molecules. L. Li, J. N. Agar, and P. Hong (co corresponding authors) Journal of the American Society for Mass Spectrometry 19(12):1867-74 (2008).

Sensitive and Specific Identification of Proteins from 8 to 669 kDa using Top-Down Mass Spectrometry. N. M. Karabacak, L. Li, A. Tiwari, L. J. Hayward, P. Hong, and J. N. Agar, Molecular and Cellular Proteomics, 8(4): 846-56. (this journal is the premier proteomics journal, with an impact factor of 8) (2009).

Tissue preparation for the in situ MALDI MS imaging of proteins, lipids, and small molecules at cellular resolution. N. Y.R. Agar, J. M. Kowalski, P. J. Kowalski, J. H. Wong, and J. N. Agar in Mass Spectrometric Imaging. History, Fundamentals and Protocols. Methods in Molecular Biology, Humana Press, Inc. Totowa, New Jersey (2009).

A Novel Strategy for Stabilizing Human Cu, Zn Superoxide Dismutase (SOD1), the Protein That Is Destabilized In the Most Common Form of Familial Amyotrophic Lateral Sclerosis. J. R. Auclair, K. J. Boggio, G. A. Petsko, D. Ringe, and J. N. Agar. In preparation, submitted to Nature Chemical Biology.

Comprehensive assignment of energy-dependent protein fragmentation reveals dissociation via three distinct fragmentation pathways. J. S. Cobb, M. L. Easterling, and J. N. Agar. In preparation, to be submitted to the Journal for the American Chemical Society.

In situ MALDI mass spectrometry imaging at cellular resolution. J.H. Wong, K. J. Boggio, N. M. Zaia, R. Lazarus, K. Sugino, S. Nelson, N. Y.R. Agar, and J. N. Agar. In preparation, to be submitted to Nature Methods.

Characterization of the Drosophila melanogaster neuropeptidome involved in circadian rhythm. K. J. Boggio, Y.W. A. Hsu, J. P. Salisbury, P.J. Kowalski, M. L. Easterling, M. Roasbash, and J. N. Agar. In preparation, to be submitted to Science.

Evaluation of methods for unsupervised classification of MALDI mass spectra from organ tissue homogenates. J. P. Salisbury, C.H. Tang, N. M. Zaia, N. Y.R. Agar, and J. N. Agar. In preparation, to be submitted to BMC Bioinformatics.

Memory Efficient Calculation of the Isotopic Mass States of a Molecule. L. Li, N. M. Karabacak, J. S. Cobb, Q. Wang, P. Hong, and J. N. Agar. In preparation, to be submitted to Journal of the American Society for Mass Spectrometry.

Multi-CHEF-ECD for high efficiency protein MS^2. N. M. Karabacak, M. L. Easterling, and J. N. Agar. In preparation, to be submitted to Journal of the American Society for Mass Spectrometry.

Transformative effects of higher magnetic field in Fourier transform mass spectrometry. N. M. Karabacak, M. L. Easterling, and J. N. Agar. In preparation, to be submitted to Journal of the American Society for Mass Spectrometry.

A comprehensive top-down mass spectrometry using Funnel-skimmer dissociation study of monoisotopic yeast proteins separated by reversed phase and hydrophilic interaction chromatography. J. S. Cobb, A. M. Morris, M. L. Easterling, and J. N. Agar. In preparation, to be submitted to Journal of the American Society for Mass Spectrometry.

Characterization of the Drosophila Melanogaster Neuropeptidome by Mass Spectrometry. K. J. Boggio, Y.W. A. Hsu, J. P. Salisbury, P. J. Kowalski, M. L. Easterling, M. Rosbash, J. N. Agar, 18th International Mass Spectrometry Conference; Bremen, Germany: Oral presentation, September 2009.

Tissue Preparation for the in situ MALDI MS Imaging of Proteins, Lipids, and Small Molecules at Cellular Resolution. N. Y.R. Agar, P. J. Kowalski, J. H. Wong, K. J. Boggio, R. M. Lazarus, J. N. Agar, 57th ASMS Conference on Mass Spectrometry and Allied Topics; Philadelphia, PA : Oral presentation, June 2009.

Optimization of MultiCHEF- and SWIFT-ECD for Protein MS^2. N. M. Karabacak, Q. Wang, C. J. Thompson, M. L. Easterling, J. N. Agar, 57th ASMS Conference on Mass Spectrometry and Allied Topics; Philadelphia, PA : Poster, June 2009.

Characterization of Drosophila melanogaster Neuropeptides by MALDI-FT-ICR Imaging MS, MALDI-TOF MS, and MALDI-TOF/TOF MS. K. J. Boggio, Y.W. A. Hsu, P. J. Kowalski, M. L. Easterling, M. R osbash, J. N. Agar, 57 th ASMS Conference on Mass Spectrometry and Allied Topics; Philadelphia, PA : Poster, June 2009.

Analysis of Cross-linked Cu/Zn-Superoxide Dismutase (SOD1) Associated with Familial Amyotrophic Lateral Sclerosis by MALDI-TOF MS and FT-ICR MS. J. R. Auclair, K. J. Boggio, G. A. Petsko, D. Ringe, J. N. Agar, 57th ASMS Confe rence on Mass Spectrometry and Allied Topics; Philadelphia, PA : Poster, June 2009.

Analysis of CS and pdf^{01} Drosophila melanogaster by Mass Spectrom etry. K. J. Boggio, J. Oh, C. J. Thom pson, M. L. Easterli ng, M. Rosbash, J. N. Agar, The Brandeis Neurogenetics Celebration; Waltham, MA: Poster, June 2008.

Optimization of Funnel-Skimm er Dissociatio n for FT-ICR Mass Spectrom etry. J. S. Cobb, M. L. Easterling, J. N. Agar, 56th ASMS Conference on Mass Spectrom etry and Allied Topics; Denver, CO: Oral Presentation, June 2008

Mass spectrom etry ap proaches to A LS biom arker D iscovery. L. Li, J. L . Johnson, D . A. Bosco, R. H. Brown Jr., N. Y.R. Agar, and J. N. Agar Treat ALS; Tampa, FL: Poster, January 2008

Discovering and Preventing Toxi c Post-Translational Modifications of SO D1. J. L. Johnson, M. Karabacak, W . Novak, G. A. Petsko, D. Ringe , and J. N. Agar Treat ALS; Tam pa, FL: Poster, January 2008.

Bibliography

Tryptophan 32 Potentiates Aggregation and Cyto toxicity of a Copper/Zinc Superoxide Dismutase Mutant Associated with Fam ilial Am yotrophic Lateral S clerosis. D. M. Taylor, B. F . Gibbs, E. Kabash i, S. Minotti, H. D. Durham , and J. N. Agar, The Journal of Biological Chemistry, 282, 16329-16335 (2007).

Matrix Solu tion Fixa tion: a Histo logy Com patible Tissu e Prepar ation for MALDI Mass Spectrometry Imaging. N. Y. R. Agar, H. W . Yang, R. S. Carroll, P. M. Black, and J. N. Agar, Analytical Chemistry, 79, 7416-7423 (2007).

Protein Agg regation an d Protein In stability Govern Fam ilial ALS Patient Surviva l. Q. Wang, J. L. Johnson, N. Y.R. Agar, and J. N. Agar. PLoS Biology. 6 (7) e170 (2008).

A Hierarch ical A lgorithm for Calculating th e Entire Is otopic Fin e Structu res of Molecules. L. Li, J. N . Agar, and P. H ong (co corresponding authors) Journal of the American Society for Mass Spectrometry 19(12):1867-74 (2008).

Sensitive and Specific Identification of Proteins from 8 to 669 kDa using Top-Down Mass Spectrometry. N. M. Karabacak, L. Li, A. Tiwari, L. J. Hayward, P. Hong, and J. N. Agar, Molecular and Cellular Proteomics, 8(4): 846-56 (this journal is the premier proteomics journal, with an impact factor of 8) (2009).

Tissue preparation for the in situ MALDI MS imaging of proteins, lipids, and small molecules at cellular resolution. N. Y.R. Agar, J. M. Kowalski, P. J. Kowalski, J. H. Wong, and J. N. Agar in Mass Spectrometric Imaging. History, Fundamentals and Protocols. Methods in Molecular Biology, Humana Press, Inc. Totowa, New Jersey (2009).

Personnel (Agar and Hong)

Jeffrey Agar: Assistant Professor
Kristin Boggio: Graduate Student
Jennifer Cobb: Graduate Student
Pengyu Hong: Assistant Professor
Murat Karabacak: Graduate Student
Joshua Kresh: Graduate student
Long Li: Postdoc
Ravi Madulla: Postdoc
Satish Murari: Postdoc
Xiaoyun Sun: Graduate student
Qi Wang: Graduate Student

Aim 8- Bioinformatics Aim: To assist sleep labs at Brandeis University with analysis of microarray data (Hong).

Introduction

Dr. Hong is specialized in bioinformatics and computational biology and was hired by Brandeis University in August 2005. Collaborating with experimental biologists, Dr. Hong and his group members are developing computational tools for (a) studying the development of neural systems and neurodegenerative diseases using high-content screen technology, (b) modeling biological networks governing animal development and signal transduction, and (c) analyzing mass spectrometry data for protein identification.

Body (Research)

(a) High-Content Image Analysis

We have developed a software imCellPhen (interactive mining of cellular phenotypes) for interactively analyzing high-content screen images (Lin, Mak et al. 2007). We reported the first full-genome RNA interference (RNAi) screen on Drosophila primary neural cells (Sepp, Hong et al. 2008). Our study revealed unexpected, essential roles in neurite outgrowth for genes representing a wide range of functional categories including signalling molecules, enzymes, channels, receptors, and cytoskeletal proteins. We also found that genes known to be involved in protein and vesicle trafficking showed similar RNAi phenotypes. We confirmed phenotypes of the protein trafficking genes Sec61alpha and Ran GTPase using Drosophila embryo and mouse embryonic cerebral cortical neurons, respectively. Collectively, our results showed that RNAi phenotypes in primary neural culture can parallel in vivo phenotypes, and the screening technique can be used to identify many new genes that have important functions in the nervous system.

(b) Biological Network Modeling

Genes and their products dynamically interact with each other at multiple levels to form complex networks that regulate how individual cells respond to environmental cues and how cells coordinate with each other via intercellular signalings to execute developmental programs. Understanding how genes/proteins regulate each other to generate functions at tissue level or a higher level can provide insights into drug therapy design, tissue engineering and regenerative medicine. My group recently took a machine learning approach and developed a methodology to infer multicellular regulatory networks by integrating heterogeneous biological data (Sun and Hong 2007; Sun and Hong 2009). We have successfully applied our approach to study *C. elegans* vulval induction, which is a paradigmatic example of animal organogenesis with extensive experimental data.

(c) Mass Spectrometry Data Analysis

In collaborating Dr. Agar of Chemistry at Brandeis, we have developed new tools for analyzing mass spectrometry data (Li, Kresh et al. 2008)(Karabacak, Li et al. 2009).

Reportable Outcomes

- Invited speaker: Symposium on Image Analysis at Harvard Medical School. April 4, 2006.
- Delegate: Frontiers in Live Cell Imaging Meeting, NIH, Natcher Conference Center, Bethesda, MD USA, April 19-21, 2006.
- Invited speaker: 2006 Workshop on Multiscale Biological Imaging, Data Mining and Informatics, Santa Barbara, CA, USA, Sept 7-8, 2006.

Conclusion

Dr. Hong's group has demonstrated its potential by developing new computational tools for systems biology research. The group will continue its research on the systematic studies of biological systems by integrating heterogeneous biological data including biological sequences, microarray data, proteomics data, and high-content images of cells. The tool developed for analyzing high-content screen image of neuron cell cultures is now being used to screen chemical compounds using primary neuron cells harvested from a *Drosophila* Huntington's disease model (collaboration with Dr. Littleton at MIT). Two chemicals were identified as potential drug candidates. In addition, Dr. Hong is now applying his biological network modeling methods to investigate the mechanism underlying *Drosophila* eye development.

References

Karabacak, N. M., L. Li, et al. (2009). "Sensitive and specific identification of wild type and variant proteins from 8 to 669 kDa using top-down mass spectrometry." Mol Cell Proteomics 8(4): 846-56.

Li, L., J. A. Kresh, et al. (2008). "A hierarchical algorithm for calculating the isotopic fine structures of molecules." J Am Soc Mass Spectrom 19(12): 1867-74.

Lin, C., W. Mak, et al. (2007). Intelligent Interfaces for Mining Large-Scale RNAi-HCS Image Databases. IEEE International Conference on Bioinformatics and Bioengineering, Harvard Medical School, Boston, MA, USA.

Sepp, K. J., P. Hong, et al. (2008). "Identification of neural outgrowth genes using genome-wide RNAi." PLoS Genet 4(7): e1000111.

Sun, X. and P. Hong (2007). "Computational modeling of Caenorhabditis elegans vulval induction." Bioinformatics 23(13): i499-i507.

Sun, X. and P. Hong (2009). "Automatic Inference of Multicellular Regulatory Networks Using Informative Priors." International Journal of Computational Biology and Drug Design 2(2): 115-133.

Personnel (Agar and Hong)

Jeffrey Agar: Assistant Professor
Kristin Boggio: Graduate Student
Jennifer Cobb: Graduate Student
Pengyu Hong: Assistant Professor
Murat Karabacak: Graduate Student
Joshua Kresh: Graduate student
Long Li: Postdoc
Ravi Madulla: Postdoc
Satish Murari: Postdoc
Xiaoyun Sun: Graduate student
Qi Wang: Graduate Student

General Conclusion

The sleep consortium at Brandeis has entered a mature phase and much of the work initiated over the past few years is reaching fruition (i.e., publication) and have produced multiple publications over the duration of this grant. The addition of Dr. Agar and Dr. Hong has greatly aided the sleep efforts at Brandeis by bringing advanced mass spectrometry and bioinformatics tools to this group, respectively.

Below is a list summarizing the publications (and 1 abstract) generated:

1. Nagoshi E., Sugino K., Kula E., Okazaki E., Tachibana T., Nelson S.B., and Rosbash M. Gene expression dissection of the circadian neuronal circuit of drosophila. Nature Neurosci. In Press.

2. PDF cells are a GABA-responsive wake-promoting component of the Drosophila sleep circuit. Parisky, K.M., Agosto, J., Pulver, S., Shang, Y., Kuklin, E., Hodge, J.L., Xu, Kang, K., Liu, X., Garrity, P., Rosbash, M., Griffith, L.C. Neuron. 2008 Nov 26; 60(4):672-82. Erratum in: Neuron. Jan 15; 61(1):152 (2009).

3. A role for microRNAs in the Drosophila circadian clock. Kadener S., Menet J., Sugino K., Horwich M.D., Nawathean P., Vagin V.V., Zamore P.D., Nelson S.B. and Rosbash M.Genes Dev. 23:2179-91 (2009).

4. Sensitive and Specific Identification of Proteins from 8 to 669 kDa using Top-Down Mass Spectrometry. N. M. Karabacak, L. Li, A. Tiwari, L. J. Hayward, P. Hong, and J. N. Agar, Molecular and Cellular Proteomics, 8(4): 846-56 (this journal is the premier proteomics journal, with an impact factor of 8) (2009).

5. Tissue preparation for the in situ MALDI MS imaging of proteins, lipids, and small molecules at cellular resolution. N. Y. R. Agar, J. M. Kowalski, P. J. Kowalski, J. H. Wong, and J. N. Agar in Mass Spectrometric Imaging. History, Fundamentals and Protocols. Methods in Molecular Biology, Humana Press, Inc. Totowa, New Jersey (2009).

6. Light-arousal and circadian photoreception circuits intersect at the large PDF cells of the Drosophila brain. Shang, Y., Griffith, L.C., Rosbash, M. Proc Natl Acad Sci U S A. 2008 Dec 16; 105(50):19587-94. Epub Dec 5 (2008).

7. Modulation of $GABA_A$ receptor desensitization uncouples sleep onset and maintenance in Drosophila. Agosto, J., Choi, J.C., Parisky, K.M., Stilwell, G., Rosbash, M., Griffith, L.C. Nat Neurosci. Mar; 11(3):354-9. Epub Jan 27 (2008).

8. Sleep: hitting the reset button. Griffith, L.C. and Rosbash, M. Nat. Neurosci. 11:123-124 (2008).

9. Protein Aggregation and Protein Instability Govern Familial ALS Patient Survival. Q. Wang, J. L. Johnson, N. Y.R. Agar, and J. N. Agar. PLoS Biology. 6 (7) e170 (2008).

10. A Hierarchical Algorithm for Calculating the Entire Isotopic Fine Structures of Molecules. L. Li, J. N. Agar, and P. Hong (co corresponding authors) Journal of the American Society for Mass Spectrometry 19(12):1867-74 (2008).

11. Non-cell-autonomous regulation of GABAergic neuron development by neurotrophins and the p75 receptor. Lin PY, Hinterneder JM, Rollor SR, Birren SJ. J Neurosci 27:12787-12796 (2007).

12. A manual method for the purification of fluorescently labeled neurons from the mammalian brain. Hempel C.M., Sugino K. and Nelson S.B Nature Protocols. 2:2924-9 (2007).

13. Tryptophan 32 Potentiates Aggregation and Cytotoxicity of a Copper/Zinc Superoxide Dismutase Mutant Associated with Familial Amyotrophic Lateral Sclerosis. D. M. Taylor, B. F. Gibbs, E. Kabashi, S. Minotti, H. D. Durham, and J. N. Agar, The Journal of Biological Chemistry, 282, 16329-16335 (2007).

14. Matrix Solution Fixation: a Histology Compatible Tissue Preparation for MALDI Mass Spectrometry Imaging. N. Y. R. Agar, H. W. Yang, R. S. Carroll, P. M. Black, and J. N. Agar, Analytical Chemistry, 79, 7416-7423 (2007).

15. Molecular taxonomy of major neuronal classes in the adult mouse forebrain. Sugino K, Hempel CM, Miller M, Hattox AM, Shapiro P, Wu C, Huang ZH and Nelson SB.Nat. Neurosci. 9:99-107 (see also News&Views p. 10-12) (2005).